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CHANGES IN THE COMPOSITION OF THE MAMMARY SECRETION OF WOMEN AFTER ABRUPT TERMINATION OF BREAST FEEDING

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SUMMARY

1. The composition of mammary secretion has been followed in seven women before and after abrupt termination of breast feeding. The period of full lactation was 39 days for 1 woman and a mean of 332 days (range 251–443 days) for the six others. Small samples of mammary secretion (0·5–5·00 ml.) were collected by manual expression from three women at monthly intervals throughout 12 months of lactation and from seven women at frequent intervals for 42 days of involution.

2. During full lactation (12 months, three women) the mean values ($\pm$ S.E. of mean) for lactose, total protein, α-lactalbumin, Na, K and Cl were $7{\cdot}03 \pm 0{\cdot}13$ g/100 ml., $1{\cdot}68 \pm 0{\cdot}08$ g/100 ml., $163 \pm 6{\cdot}39$ mg/100 ml., $8{\cdot}5 \pm 0{\cdot}90$ mM, $13{\cdot}4 \pm 0{\cdot}34$ mM and $11{\cdot}93 \pm 0{\cdot}53$ mM, respectively.

3. After termination of breast feeding, the concentrations of lactose and K decreased while Na, Cl, fat and total protein increased progressively over 42 days. The increase in the protein content was contributed to by increases in the concentration of lactoferrin, IgA, IgG, IgM, albumin, α-lactalbumin and casein. There was no significant difference in the concentration of the milk constituents between the right and left breast throughout full lactation and after its termination.

4. These observations indicated that the secretory capability of the mammary gland of women changed dramatically after complete cessation of breast feeding but that the involuting gland remained partially functional for a long period.

INTRODUCTION

Studies on dairy animals have shown that the composition of mammary secretion before parturition and after the termination of an established lactation, is different from that of normal milk (Linzell & Peaker, 1971*a*). In the cow, the concentrations of fat, proteins, Na and Cl are higher while lactose and K are lower in the colostrum than in the normal milk (Linzell & Peaker, 1971*a*). Similar changes have been reported to occur in the mammary secretion of women during the onset of lactation (Gunther, Hawkins & Whyley, 1965; Lönnerdal, Forsum & Hambraeus, 1976; Peaker, 1976; Kulski, Smith & Hartmann, 1977) but little is known about the variation of these milk constituents during the period of mammary involution. This study was undertaken to determine the progressive changes of the milk constituents in the mammary secretion of women during an established lactation and after abrupt termination of breast feeding.

METHODS

Collection of mammary secretion

The details of seven healthy women who provided samples of mammary secretion before and after the termination of an established lactation are outlined in Table 1. All the women had normal deliveries and felt that adequate volumes of milk were produced to feed their infants throughout lactation.

Since all the women weaned their infants abruptly, the onset of involution (day 1) represents the changeover from breast feeding to complete cessation of suckling. Breast stimulation during involution was limited to the collection of small samples of mammary secretion.

TABLE 1. Details of women studied

Subject no.	Age	Sex of child breast fed	Lactational length (days after birth)
W1	23	F	291
W2	25	M	251
W3	29	M	263
W4	30	M	328
W5	30	F	411
W6	33	M	443
W7	23	F	39

F = female. M = male.

Samples of mammary secretion were obtained from one woman (W7) at 15 days before and at 0, 11, 22, 27, 35 and 45 days after she had stopped breast feeding 39 days after delivery. The average period of full lactation was 332 days (range 251–443 days) for the other six women (W1–W6). Samples of mammary secretion were obtained from these women at 22 and 7 days before and at 1, 2, 4, 8, 16, 21, 31 and 42 days after termination of breast feeding. In one woman (W6), secretion was expressed from the breasts at 64 days of involution whereas in the other women further secretion could not be expressed after 45 days. Samples were also obtained from three of the women (W4–W6) at monthly intervals throughout 12 months of lactation.

Each woman collected her own samples (0.5–5·0 ml.) from each breast by manually expressing the secretion directly into sterile polypropylene vials. Sampling was carried out between 10.00 and 12.00 noon on each day of the collection and the samples were stored at -15 °C until analysed.

Analysis of mammary secretion

Lactose, in a 1:100 dilution (v/v) of mammary secretion in water, and glucose in the whey fraction of the mammary secretion, were measured by the method of Kuhn & Lowenstein (1967). Since the glucose content of the mammary secretion of women never exceeded 40 mg/100 ml., no correction was made for glucose present before hydrolysis of lactose. This procedure resulted in a maximum over-estimation of lactose of $< 1{\cdot}0\,\%$.

Total protein was estimated by a micro-Kjeldahl method using the distillation apparatus designed by Leurquin & Delville (1950). Milk protein was calculated as total N $\times$ 6·38 (Hartmann, 1973). Na and K in a 1:100 dilution (v/v) of mammary secretion in 150 mM-Li_2SO_4 were measured by flame photometry (EEL 450 (Evans Electroselenium Ltd)) using the Li_2SO_4 solution as an internal reference. Cl was measured by coulimetric titration (Radiometer CMT 10 chloride titrator, Radiometer Electronic Measuring Instruments). Milk fat was estimated as total esterified fatty acids (Hartmann, 1973) assuming the mean molecular weight of milk fatty acid of the human to be 265 (Garton, 1963). Casein was estimated as phosphoprotein precipitated by 10 % trichloroacetic acid (w/v). Phosphorus was determined by the method of Zilversmit & Davis (1950) and the protein concentration was calculated assuming that casein contained 0·45 % phosphorus (Jolles, 1966). α-Lactalbumin, lactoferrin, serum albumin, IgA, IgG and IgM were determined by the single radial immunodiffusion technique of Mancini, Carbonara & Heremans (1965).

Human α-lactalbumin was purified by the procedure of Phillips & Jenness (1971) and this preparation was active with galactosyl transferase in the lactose synthetase reaction (Ebner, Mawal, Fitzgerald & Colvin, 1972). The concentration of α-lactalbumin was determined by measuring the optical density at 280 nm assuming a molar extinction of 20,400 and molecular weight of 14,081 (Phillips & Jenness, 1971). Human lactoferrin was purified by the procedure of Querinjean, Masson & Heremans (1971) and the preparation was standardized by weighing freeze dried samples. α-Lactalbumin and lactoferrin each migrated as a single band on polyacrylamide gel disk electrophoresis (pH 8·6) and gave a single precipitin line against anti-human milk whey. Rabbit antiserum to either α-lactalbumin or lactoferrin gave a single precipitin line against human milk whey. Standard solutions of, and specific antisera to, human serum albumin, IgA, IgG and IgM were obtained from Behringwerke AG, Marburg, W. Germany.

RESULTS

Changes in the composition of the mammary secretion

During lactation

The mean monthly changes in the concentration of lactose, α-lactalbumin, total protein, Na, K and Cl in the mammary secretion from the right and left breasts of three women (W4–W6) during 12 months of lactation are shown in Fig. 1. Over the 12 months of lactation the mean concentrations (± S.E. of mean) of lactose, total protein, α-lactalbumin, Na, K and Cl were 7·03 ± 0·13 g/100 ml., 1·68 ± 0·08 g/100 ml., 163·40 ± 6·39 mg/100 ml., 8·50 ± 0·90 mM, 13·40 ± 0·34 mM and 11·93 ± 0·53 mM, respectively. There were significant ($P < 0{\cdot}05$) negative correlations between the changes in the concentration of lactose and Na ($r = -0{\cdot}29$, $n = 60$), lactose and K ($r = -0{\cdot}29$, $n = 60$) and lactose and Cl ($r = -0{\cdot}33$, n = 58) and significant positive correlations between K and Cl ($r = 0{\cdot}33$, $n = 58$) and K and Na ($r = 0{\cdot}32$, $n = 60$). No significant ($P > 0{\cdot}05$) correlations were obtained between the concentrations of lactose and protein ($r = 0{\cdot}13$, $n = 60$) and lactose and α-lactalbumin ($r = -0{\cdot}23$, $n = 60$). Moreover, there was no significant difference in the concentration of the milk constituents between the right and left breast throughout lactation.

During involution after 11 months lactation

Lactose, total protein and fat. The mean changes in the concentration of lactose, total protein and fat in the mammary secretion of six women (W1–W6) during involution are shown in Fig. 2*A*. Whereas, the concentration of lactose decreased fivefold during the first 42 days of involution, the concentration of total proteins increased sixfold during the same period. In contrast to lactose and total protein, the total esterified fatty acids did not change significantly during the first 8 days (range 3·9–4·1 g/100 ml.), but thereafter increased 2·5-fold.

There were significant ($P < 0{\cdot}05$) negative correlations between lactose and protein ($r = -0{\cdot}80$, $n = 92$) and lactose and total esterified fatty acids ($r = -0{\cdot}35$, $n = 56$).

Glucose. The concentration of glucose in the mammary secretion was high (range 20–42 mg/100 ml.) over lactation and then fell abruptly to lower levels (range 1·0–5·0 mg/100 ml.) during involution.

Na, K and Cl. The mean changes in the concentration of Na, Cl, K in the mammary secretion of six women (W1–W6) during involution are shown in Fig. 2*B*. The

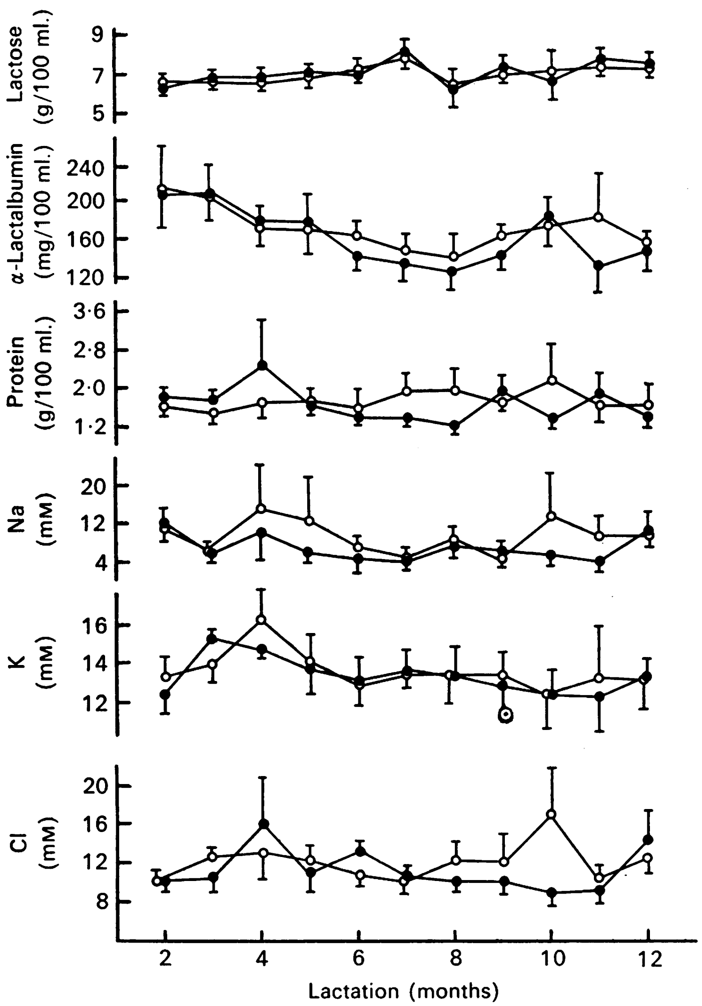

Fig. 1. Changes in the concentration of lactose, α-lactalbumin, total protein, Na, K and Cl in the mammary secretion of the right ●, and left ○, breasts of three women (W4–W6) during the first 12 months of lactation (mean ± S.E. of mean).

concentrations of Na and Cl increased 3.8-fold and 2.4-fold, respectively, while K decreased 2·4-fold during the first 42 days of involution. However, the greatest changes in the concentration of Na, Cl and K occurred during the first 8 days of involution. There were significant ($P < 0{\cdot}05$) negative correlations between the lactose and Na ($r = -0{\cdot}90$, $n = 101$), lactose and Cl ($r = -0{\cdot}77$, $n = 94$) and K and Na ($r = -0{\cdot}77$, $n = 101$) and significant ($P < 0{\cdot}05$) positive correlations between lactose and K ($r = 0{\cdot}82$, $n = 101$) and Na and Cl ($r = 0{\cdot}91$, $n = 94$).

IgA, IgG, IgM and α-lactalbumin. The changes in the concentration of IgA, IgG, IgM and α-lactalbumin in the mammary secretion of four women (W1–W4) during involution are shown in Fig. 3*A*. Whereas IgG increased 8·2-fold over the first 42 days after termination of breast feeding, the concentration of IgM and IgA increased twentyfold and fivefold, respectively. The concentration of α-lactalbumin increased twofold over the first 21 days and then decreased slightly (261–237 mg/100 ml.) over the next 21 days. However, there was a significant ($P < 0{\cdot}05$) negative correlation between lactose and α-lactalbumin ($r = -0{\cdot}52$, $n = 63$) and a significant ($P < 0{\cdot}05$) positive correlation between α-lactalbumin and total protein content ($r = 0{\cdot}61$ $n = 63$) over the 42 days of involution. Furthermore, there were significant ($P < 0{\cdot}05$) negative correlations ($r < -0{\cdot}80$, $n > 22$) between lactose and the immunoglobulins, IgA, IgG and IgM.

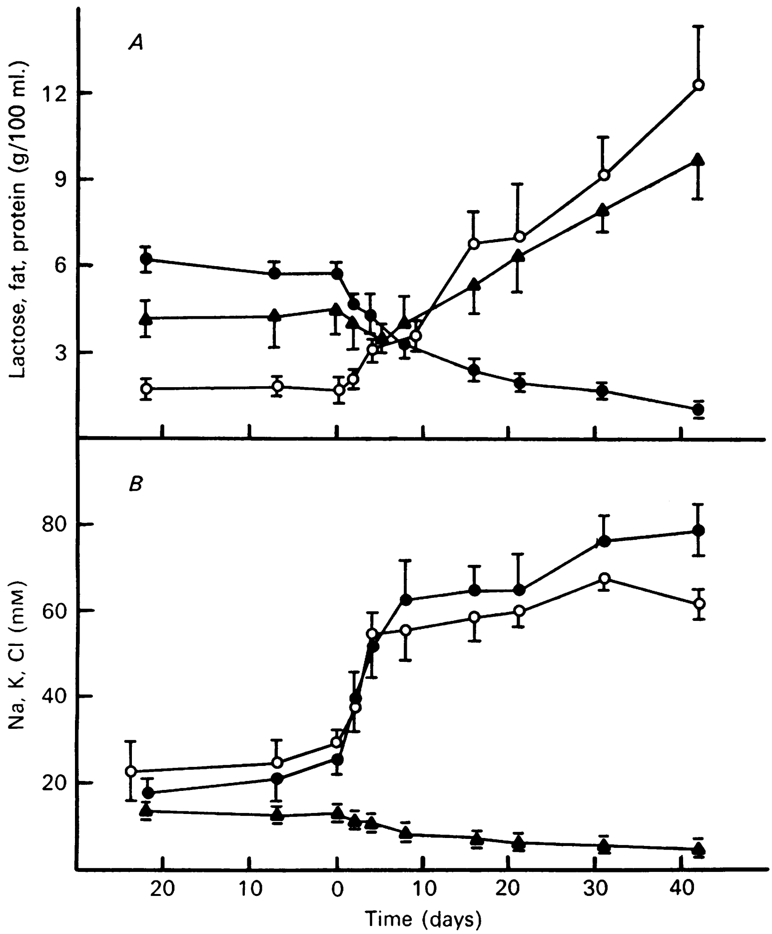

Fig. 2. Changes in the concentration of (*A*) lactose, ●; total protein, ○; fat, ▲; (*B*) Na, ●; K, ▲; Cl, ○ in the mammary secretion of six women (W1–W6) from 22 days before until 42 days after termination of breast feeding (values obtained for the right and left breast for each woman were averaged and used to calculate the mean ± S.E. of mean at each time). Zero on abscissa indicates the time that breast feeding was terminated.

Casein, serum albumin and lactoferrin. The changes in the concentrations of casein, serum albumin and lactoferrin in the mammary secretion of one woman (W3) during involution are shown in Fig. 3*B*. The concentrations of lactoferrin, serum albumin and casein increased ten, eight and fourfold respectively, during the first 42 days of involution. There were significant ($P < 0{\cdot}05$) negative correlations ($r < -0{\cdot}80$,

$n > 20$) between lactose and the protein components, lactoferrin, serum albumin and casein.

To determine the relation between the protein components and total protein during involution in this woman (W3) the protein values obtained 7 days before and 42 days after the termination of breast feeding were expressed as a percentage of total protein (Fig. 4). An increase in the proportion of IgG, IgM, lactoferrin and serum albumin and a decrease in the proportion of casein, α-lactalbumin and IgA over the period of involution was observed.

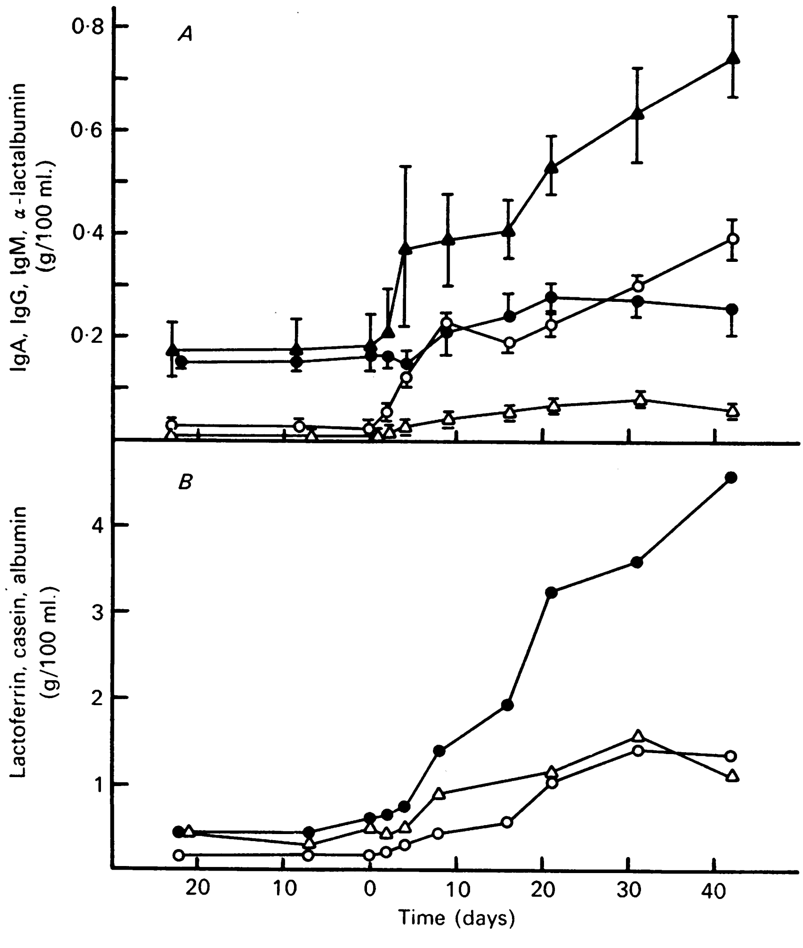

Fig. 3. Changes in the concentration of (*A*) IgA ▲, IgG △, IgM ○ and α-lactalbumin ● in the mammary secretion of 4 women (W1–W4) and (*B*) lactoferrin ●, albumin ○ and casein △ in the mammary secretion of one woman (W3) from 22 days before until 42 days after termination of breast feeding (values obtained for the right and left breast for each woman were averaged and used to calculate the mean ± S.E. of mean at each time). Zero on abscissa indicates the time that breast feeding was terminated.

During involution after 39 day lactation

The changes in the concentration of Na, Cl, K, lactose, total protein and α-lactalbumin in the mammary secretion of the right and left breast of one woman (W7) who ceased to breast feed her infant after an established lactation of 39 days are shown in Fig. 5. In this woman, as in all of the women studied, there was close agreement between the right and left breasts in the concentration of the individual milk constituents. These changes were similar to those previously described for six women (W1–W6) who had terminated breast feeding after more than 251 days of lactation (Figs. 2 and 3).

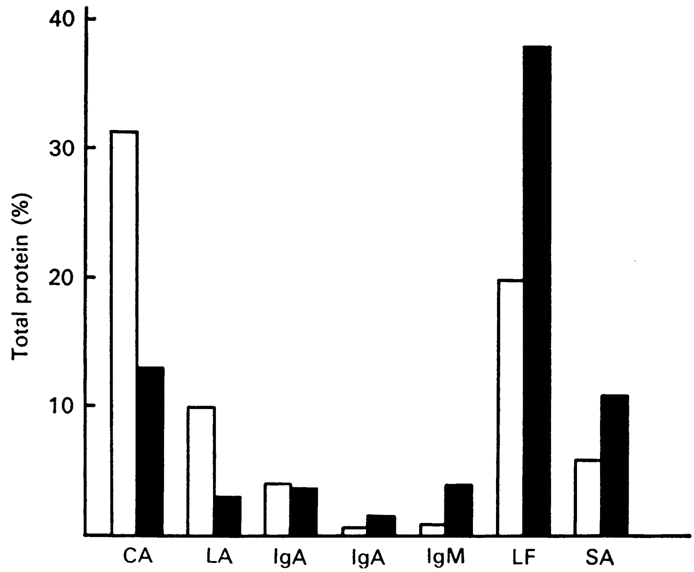

Fig. 4. Casein (CA), α-lactalbumin (LA), IgA, IgG, IgM, lactoferrin (LF) and serum albumin (SA) as a percentage of the total protein 7 days before (open bars) and 42 days after (filled bars) the termination of breast feeding in one woman (W3).

DISCUSSION

The average concentrations of lactose, Na, K, Cl, total protein and α-lactalbumin during established lactation (Fig. 1) were in close agreement with the results of other workers who have measured one or other of these constituents (Macy, Kelly & Sloan, 1953; Ramadan, Salah, Eid & Sammour, 1972; Lönnerdal, Forsum & Hambraeus, 1976). Although the month-to -month levels of these constituents remained relatively stable over 12 months of lactation, there was a significant negative correlation between lactose and K. This relation, which also has been observed in the milk composition of goats and cows during full lactation (Peaker, 1975), indicates that the movement of lactose and ions from the intracellular fluid of the secretory cell to the milk occurred mainly by way of a transcellular pathway (Linzell & Peaker, 1971*a*; Peaker, 1976).

However, with the onset of involution, the concentrations of lactose, glucose and K decreased progressively while those of Na and Cl increased (Fig. 2). Similar relations have been observed during declining lactation in the rabbit (Peaker & Taylor, 1975) and the guinea-pig (Peaker, Jones, Goode & Linzell, 1975), involution in cows (Wheelock, Smith, Dodd & Lyster, 1967; Hartmann, 1973) and oxytocin treatment (removal of residual milk) in the rabbit (Linzell, Peaker & Taylor, 1975) and the goat

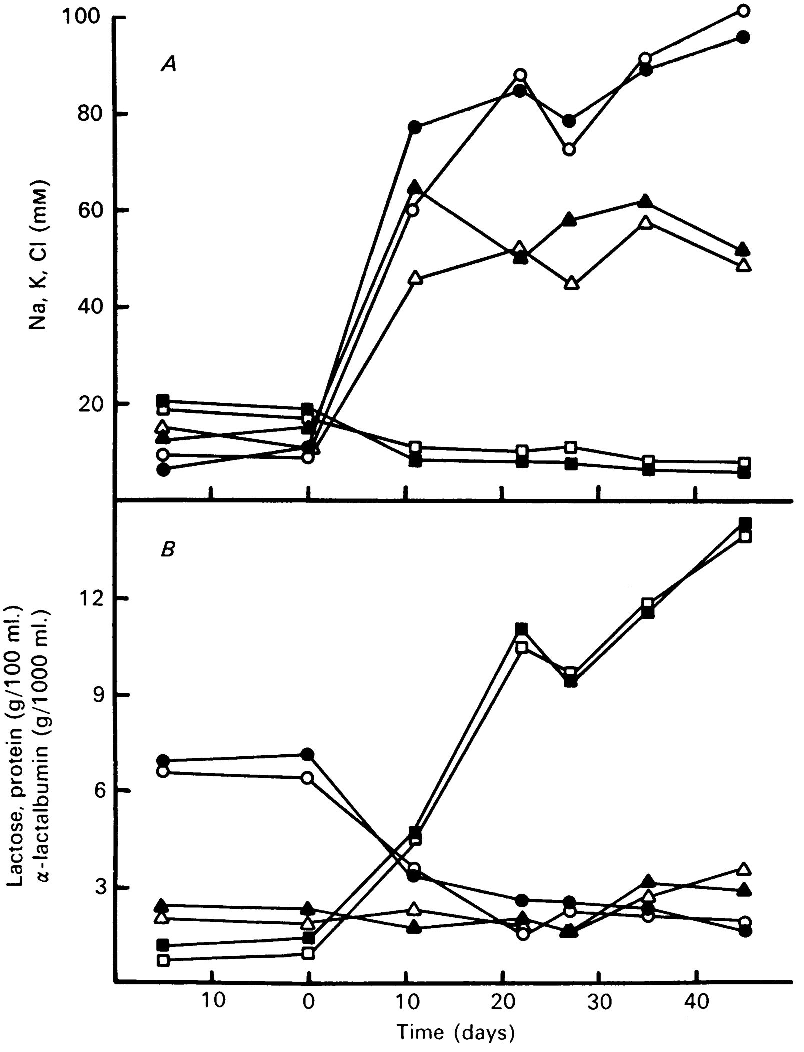

Fig. 5. Changes in the concentration of (*A*) Na, ●; Cl, ▲; K, ■; (*B*) lactose, ●; total protein, ■; and α-lactalbumin, ▲, in the mammary secretion of the right (open symbols) and left breast (filled symbols) of one woman (W.7) from 0 to 45 days of involution. This woman breast fed for 39 days. Zero on abscissa indicates the time that breast feeding was terminated.

(Linzell & Peaker, 1971*b*). These changes appear to indicate an increased permeability by a paracellular pathway (i.e. 'leaky' tight junctions of the cells) in the mammary gland (Linzell & Peaker, 1974; Peaker & Taylor, 1975; Peaker, 1976) which allows the exchange of lactose and ions between the extracellular fluid and milk. Similarly, the inverse relation observed in the present study, between the fall in lactose (Fig. 1) and the increase in all the protein components (Fig. 3) is consistent with the existence of a paracellular route in the mammary glands of women during involution. It would seem that lactose is reabsorbed from the mammary secretion by the paracellular route at a faster rate than the proteins, which tend to be retained in the mammary secretion when the tight junctions are not sufficiently leaky to allow the passage of proteins in significant amounts (Linzell & Peaker, 1974). Thus, the changes in the composition of the aqueous phase of human mammary secretion may be explained by the same mechanisms that have been proposed for other species (see Peaker, 1976). However, ultrastructural studies of the tight junction of the epithelial alveoli cell in the human are required to confirm that there is a change in the form of the tight junctions during involution.

Although the concentration of all the proteins increased during involution (Fig. 3) there were differences in the rate at which these proteins changed (Fig. 4). The proteins synthesized by the alveolar cells (α-lactalbumin and caseins) were found to decrease proportionally more than the proteins derived from serum (IgG, IgM and serum albumin). Since the proportion of total protein contributed by lactoferrin increased at a rate similar to that of the serum derived proteins, the synthesis and secretion of lactoferrin is probably regulated by a process which differs from that which controls the synthesis and secretion of α-lactalbumin and caseins. It is possible that lactoferrin is synthesized by the mammary epithelium (Loisillier, Got, Burtin & Grabar, 1966) and also by neutrophils (Masson, Heremans & Schonne, 1969) which can infiltrate the interstitial tissues during involution (Mayer & Klein, 1961).

One of the main roles of lactoferrin (Goldman & Smith, 1973) and the immunoglobulins (Hanson, Carlsson, Ahlstedt, Svanborg & Kaijser, 1975) in the mammary secretion is clearly antimicrobial. Therefore, the increased content of these proteins during involution (Fig. 3) provides protection to the mammary gland against infection at a time when milk stasis in the gland favours bacterial growth. The high content of the antibacterial proteins would also favour those infants who, because of illness or injury, return to the breast during normal weaning.

In comparison to the mammary glands of cows (Hartmann, 1973), sows and rats (K. Nicholas, C. Martin & P. E. Hartmann, unpublished observations) which can resorb milk components completely within approximately 7 days, the involuting gland of women remained functional for a long period, with secretion still being present up to 42 days after the abrupt termination of breast feeding. The observation that involution of the breasts of women is relatively gradual after termination of breast feeding is consistent with the findings of Caldeyro-Barcia (1969) who showed that the threshold dose of oxytocin required to elicit a milk ejection response in women increases progressively for at least 30 days after termination of breast feeding. The cause and the physiological significance for this variation between humans and other species is now known, but clearly warrants further study. It is possible that a psychological nursing stimulus may have partly contributed to prolonging the involutional process, since in contrast to animal studies, mother and child were not separated from each other after breast feeding was terminated. In women (Caldeyro-Barcia, 1969) and cows (Ceverley, 1968) a conditioned stimulation can produce a reflex release of oxytocin. This hormone, has been found to delay involution in cows

(Gorman & Swanson, 1968) and rats (Benson & Folley, 1957) when administered after cessation of suckling.

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REFERENCES

BENSON, G. K. & FOLLEY, S. J. (1957). The effect of oxytocin on mammary gland involution in the rat. *J. Endocr.* **16**, 189–201.

CALDEYRO-BARCIA, R. (1969). Milk ejection in women. In *Lactogenesis: the Initiation of Milk Secretion at Parturition*, ed. REYNOLDS, M. & FOLLEY, S. J., pp. 229–243. Philadelphia: University of Pennsylvania Press.

CEVERLEY, J. D. (1968). The detection of oxytocin release in response to conditioned stimuli associated with machine milking in the cow. *J. Endocr.* **40**, ii–iii.

EBNER, K. E., MAWAL, R., FITZGERALD, D. K. & COLVIN, B. (1972). α-Lactalbumin and the synthesis of lactose. *Meth. Enzym.* **28** (B), 507–510.

GARTON, G. A. (1963). The composition and biosynthesis of milk lipids. *J. Lipid Res.* **4**, 237–254.

GOLDMAN, A. S. & SMITH, C. W. (1973). Host resistance factors in human milk. *J. Pediat.* **82**, 1082–1090.

GORMAN, G. M. & SWANSON, E. W. (1968). Effects of oxytocin administration during the dry period on the succeeding lactation. *J. Dairy Sci.* **51**, 60–66.

GUNTHER, M., HAWKINS, D. T., & WHYLEY, G. A. (1965). Some observations on the sodium and potassium content of human milk. *J. Obstet. Gynaec. Br. Commonw.* **72**, 69–74.

HANSON, L. A., CARLSSON, B., AHLSTEDT, S., SVANBORG, C. & KAIJSER, B. (1975). Immune defence factors in human milk. *Modern Problems in Paediat.* **15**, 63–72.

HARTMANN, P. E. (1973). Changes in the composition and yield of the mammary secretion of cows during the initiation of lactation. *J. Endocr.* **59**, 231–247.

JOLLES, P. (1966). Casein. In *Glycoproteins: their Composition, Structure and Function*, BBA Library vol. 5, ed. GOTTSCHALK, A. P., pp. 335–352. Amsterdam: Elsevier.

KUHN, N. J. & LOWENSTEIN, J. M. (1967). Lactogenesis in the rat. Changes in metabolic parameters at parturition. *Biochem, J.* **105**, 995–1002.

KULSKI, J. K., SMITH, M. & HARTMANN, P. E. (1977). Perinatal concentrations of progesterone, lactose and α-lactalbumin in the mammary secretion of women. *J. Endocr.* **74**, 509–510.

LEURQUIN, J. & DELVILLE, J. P. (1950). Modification de l'appareil à aération de Van Slyke – Cullen et son adaption au microdosage de l'azote protéique après Kjeldahlisation et entrainement à froid de l'ammoniaque. *Experientia* **6**, 274–275.

LINZELL, J. L. & PEAKER, M. (1971*a*) Mechanism of milk secretion. *Physiol. Rev.* **51**, 564–597.

LINZELL, J. L. & PEAKER, M. (1971*b*). The effects of oxytocin and milk removal on milk secretion in the goat. *J. Physiol.* **216**, 717–734.

LINZELL, J. L. & PEAKER, M. (1974). Changes in colostrum composition and in the permeability of the mammary epithelium at about the time of parturition in the goat. *J. Physiol.* **243**, 129–151.

LINZELL, J. L., PEAKER, M. & TAYLOR, J. C. (1975). The effects of prolactin and oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. *J. Physiol.* **253**, 547–563.

LOISILLIER, F., GOT, R., BURTIN, P. & GRABAR, P. (1966). Recherches sur la localisation tissulaire et l'auto-antigènicité de la lactotransferrine. *Prot. biol. Fluids.* **14**, 133–142.

LÖNNERDAL, B., FORSUM, E. & HAMBRAEUS, L. (1976). A longitudinal study of the protein, nitrogen and lactose contents of human milk from Swedish well-nourished mothers. *Am. J. clin. Nutr.* **29**, 1127–1133.

MACY, I. G., KELLY, H. J. & SLOAN, R. E. (1953). The composition of milks. *Nat. Acad. Sci., Nat. Res Counc. Publ.*, vol. 254, pp. 25–38: Washington, D.C.

MANCINI, G., CARBONARA, P. O. & HEREMANS, J. I. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* **2**, 235–254.

MASSON, P. L., HEREMANS, J. F. & SCHONNE, E. (1969). Lactoferrin, an iron binding protein in neutrophilic leukocytes. *J. exp. Med.* **130**, 643–658.

MAYER, G. & KLEIN, M. (1961). Histology and cytology of mammary gland. In *Milk: the Mammary Gland and its Secretion*, vol. 1, ed. KON, S. K. & COWIE, A. T., pp. 47–126. London: Academic.

PEAKER, M. (1975). Recent advances in the study of monovalent ion movements across the mammary epithelium: relation to onset of lactation. *J. Dairy Sci.* **58**, 1042–1047.

PEAKER, M. (1976). Lactation: some cardiovascular and metabolic consequences, and the mechanisms of lactose and ion secretion into milk. In *Breast-feeding and the Mother*, Ciba Foundation Symposium no. 45. Amsterdam: Elsevier/Excerpta Medica/North Holland.

PEAKER, M., JONES, C. D. R., GOODE, J. A. & LINZELL, J. L. (1975). Changes in milk composition during lactation in the guinea pig, and the effects of prolactin. *J. Endocr.* **67**, 307–308.

PEAKER, M. & TAYLOR, J. C. (1975). Milk secretion in the rabbit: changes during lactation and the mechanism of ion transport. *J. Physiol.* **253**, 527–545.

PHILLIPS, N. I. & JENNESS, R. (1971). Isolation and properties of human α-lactalbumin. *Biochim. biophys. Acta* **229**, 407–410.

QUERINJEAN, P., MASSON, P. L., & HEREMANS, J. I. (1971). Molecular weight, single-chain structure and amino acid composition of human lactoferrin. *Eur. J. Biochem.* **20**, 420–425.

RAMADAN, M. A., SALAH, M. M., EID, S. Z. & SAMMOUR, M. B. (1972). The effect of the oral contraceptive Ovosiston on the composition of human milk. *J. reprod. Med.* **9**, 81–83.

WHEELOCK, J. V., SMITH, A., DODD, F. H. & LYSTER, R. L. J. (1967). Changes in the quantity and composition of mammary gland secretion in the dry period between lactations. 1. The beginning of the dry period. *J. Dairy Res.* **34**, 1–12.

ZILVERSMIT, D. B. & DAVIS, A. K. (1950). Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J. Lab. clin. Med.* **35**, 155–160.